

Characterization of the functional variance of MbtH-like protein interactions with nonribosomal peptide synthetases.

Supporting Information.

Rebecca A. Schomer[†] and Michael G. Thomas^{†*}

[†]Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, 53706.

* Corresponding Author: Contact information for the author to whom correspondence should be addressed. Phone: (608) 263-9075 Email: michael.thomas@wisc.edu

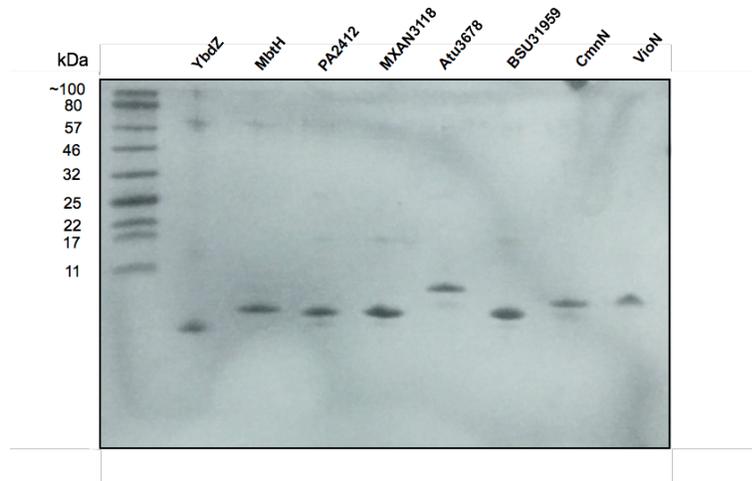


Figure S1. Purified MLPs: 2.5 μg of purified MLP separated by 16.5% polyacrylamide tris-tricine gel.

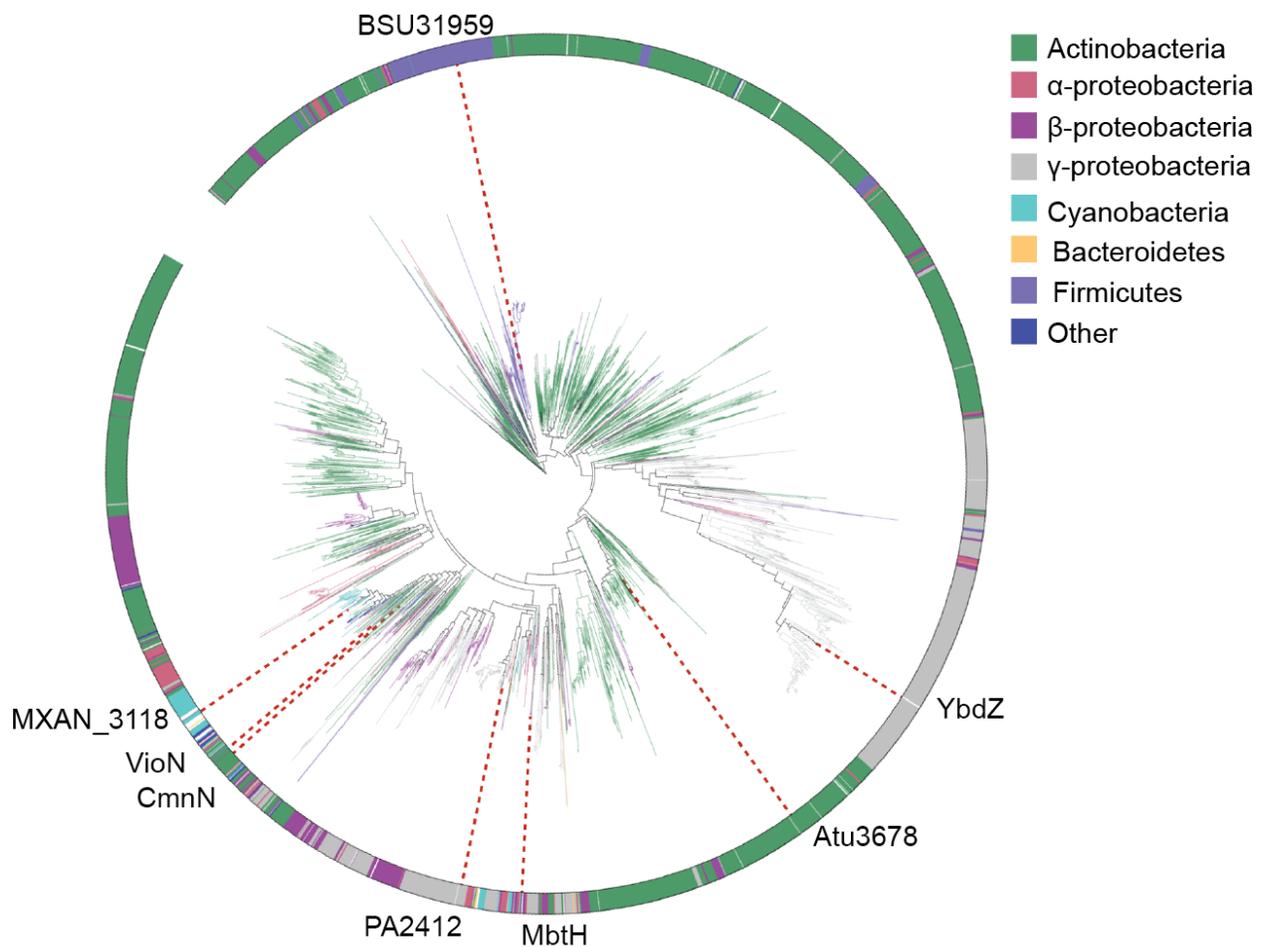


Figure S2. Phylogeny of MbtH-like proteins. A phylogeny of MLPs was generated from 5,393 unique predicted MLPs generated from sequenced genomes in GenBank including 3,054 Actinobacteria, 164 α -proteobacteria, 451 β -proteobacteria, 1,168 γ -proteobacteria, 312 Firmicutes, 98 Cyanobacteria, 7 Bacteroidetes, 6 Chloroflexi 2 Acidobacteria, 4 Nitrospirae, 2 Planctomycetes, 1 Chlamydiae, 1 Calditrichaeota and 23 metagenomic samples. Dashed red lines indicate the location of each MLP used in this study.

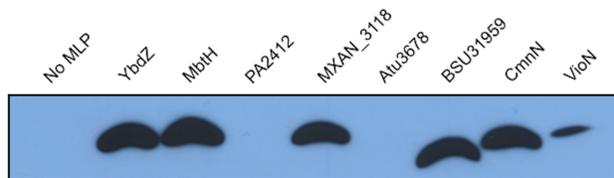


Figure S3. Representative immunoblot analysis of levels of MLPs-T7 in *E. coli* BW27749 $\Delta ybdZ$. Cells were grown in ILM with glucose as the carbon source. Once cells reached an OD_{600} of 0.5, arabinose was introduced to increase expression of the MLP-T7 genes. Cells were harvested 1 hr after arabinose addition.

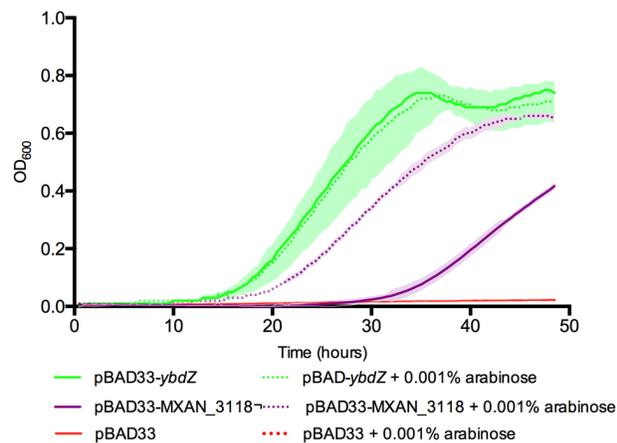


Figure S4. Analysis of $\Delta ybdZ$ complementation by increased expression of MXAN_3118 by addition of 0.001% arabinose to media. Data represented are the means with standard deviations from experimental triplicates.

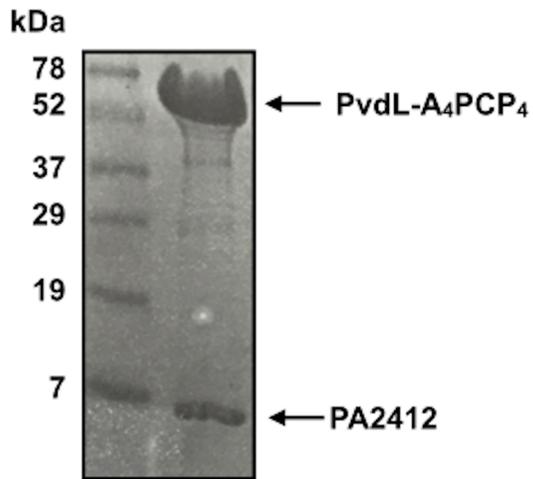


Figure S5. Co-production and purification of PvdL-A₄PCP₄ with PA2412 on 15% polyacrylamide gel.

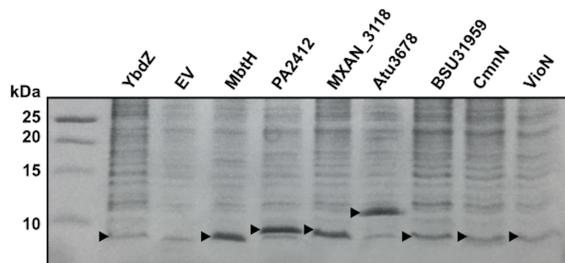


Figure S6. Analysis of production of soluble MLPs when co-produced with EntF by *E. coli* BL21(de3) $\Delta ybdZ$. Equal amounts of total protein (75 μ g) were loaded for each sample on a tris-tricine 16.5% acrylamide gel.

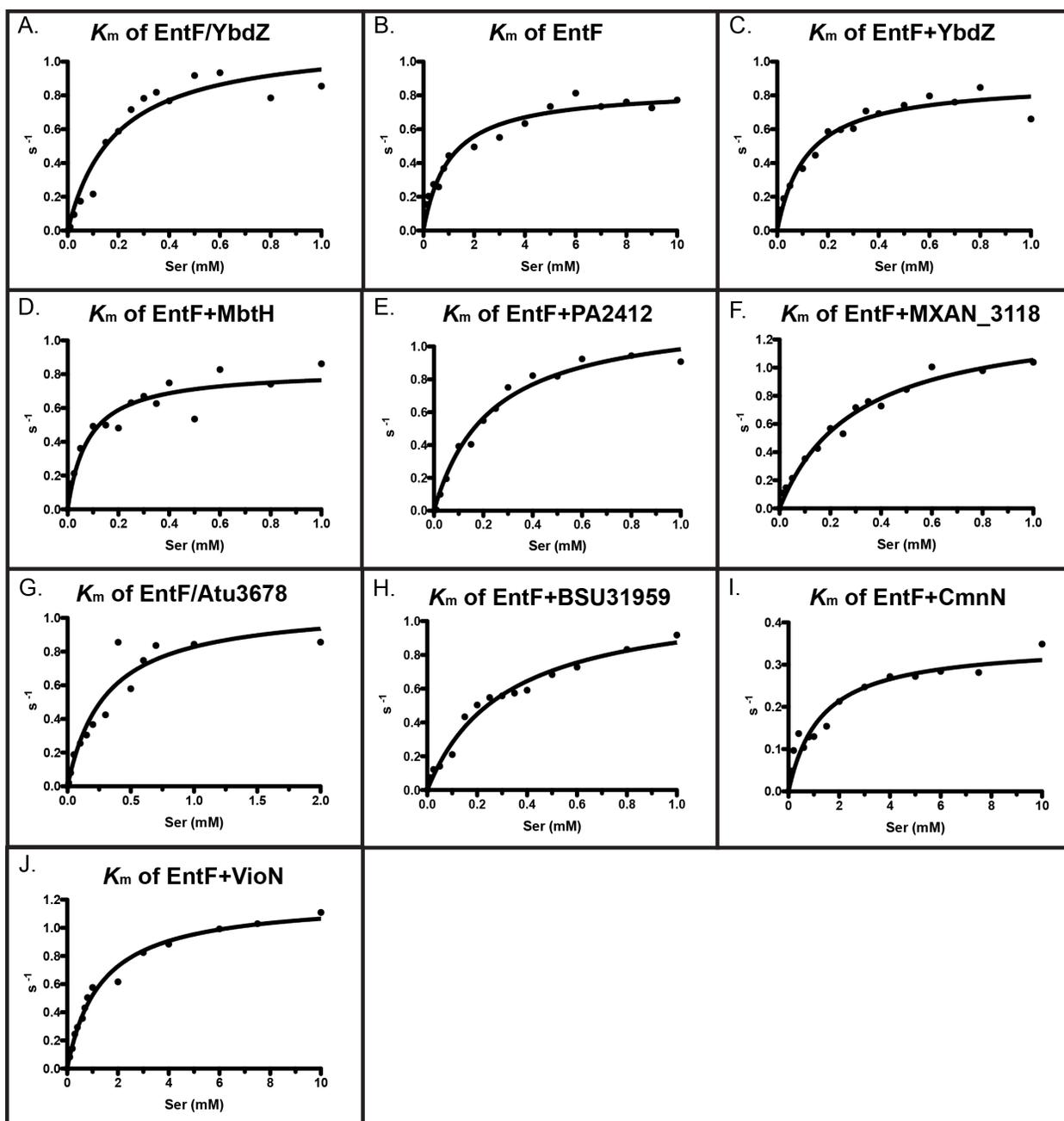


Figure S7. Kinetic analysis of L-Ser activation by EntF copurified with YbdZ (A) or Atu3678 (G), purified without an MLP (B) and in the presence of separately purified YbdZ (C), MbtH (D), PA2412 (E), MXAN_3118 (F), BSU31959 (H), CmnN (I) or VioN (J). Each panel is a representative of multiple trials. All assays were performed in the linear range of enzyme concentration and less than 10% substrate to product conversion.

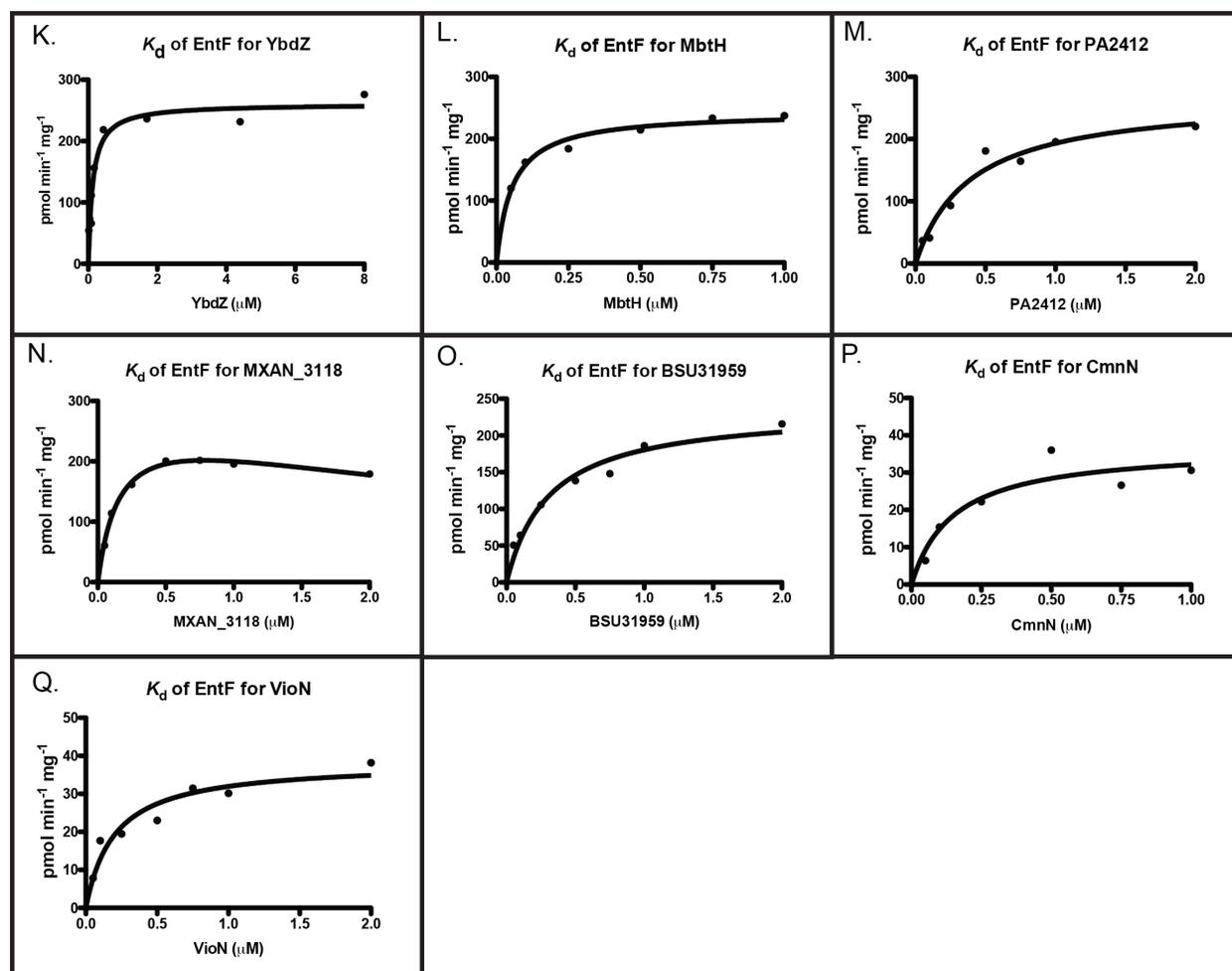


Figure S8. Representative kinetic analyses of L-Ser activation by EntF with varying concentrations of YbdZ (K), MbtH (L), PA2412 (M), MXAN_3118 (N), BSU31959 (O), CmnN (P) or VioN (Q). All assays were performed in the linear range of enzyme concentration and less than 10% substrate to product conversion.

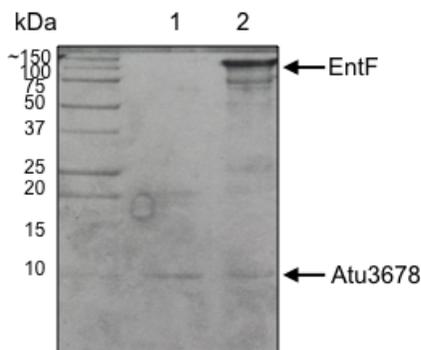


Figure S9. Analysis of overproduced EntF in the presence of Atu3678: 1 µg of Atu3678 (lane 1) 2 µg of EntF/Atu3678 (lane 2) separated by 16.5% polyacrylamide tris-tricine gel.

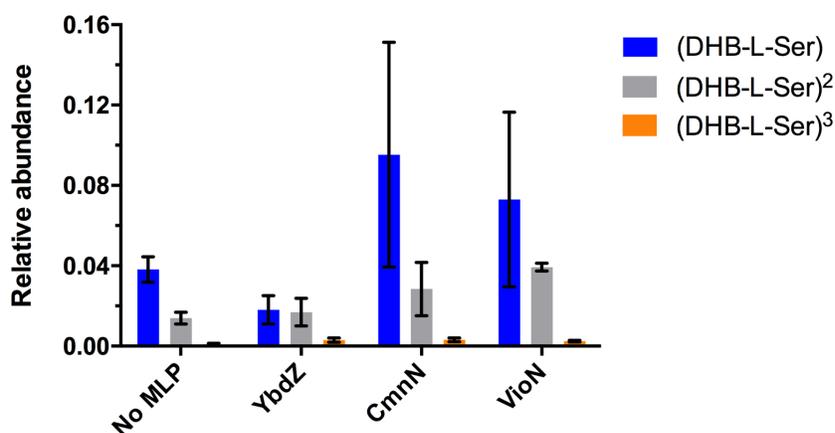


Figure S10. Comparisons of ENT, (DHB-L-Ser), (DHB-L-Ser)² and (DHB-L-Ser)³ in the presence and absence of YbdZ, CmnN or VioN. For each sample, ENT detection was set to 1. Though ENT, (DHB-L-Ser), (DHB-L-Ser)², and (DHB-L-Ser)³ are detected at different efficiencies in MS, we assume that the difference in detection efficiency to ENT remains consistent across samples. Therefore, within each sample, the area of detection for (DHB-L-Ser), (DHB-L-Ser)², and (DHB-L-Ser)³ was compared directly to the area of detection of ENT in that sample to determine the relative abundance of each intermediate. The average of the ratios from triplicate LC/MS samples is reported with standard deviation. These ratios were then compared across samples to determine if there was a significant skew for a specific intermediate within a data set. Ultimately, a robust comparison of intermediates formed in the reaction was impaired by low detection rates and an inability to directly quantify and compare amount of each intermediate in a sample. As expected with the low detection of the intermediates in comparison to ENT in all samples, variation in the samples resulted in greater error and no statistically distinct differences.

Table S1. Strains used in this study. Species is *Escherichia coli* unless otherwise noted.

Strains:	Purpose or relevant characteristics:	Source:
DH5 α	<i>fhuA2</i> $\Delta(\text{argF-lacZ})$ U169 <i>phoA</i> <i>glnV44</i> Φ 80 $\Delta(\text{lacZ})$ M15 <i>gyrA96</i> <i>recA1</i> <i>relA1</i> <i>endA1</i> <i>thi-1</i> <i>hsdR17</i>	laboratory strain
BL21(DE3) <i>ybdZ::acc(3)IV</i>	BL21(DE3) with apramycin resistance gene disrupting <i>ybdZ</i>	1
BW27749	F-, $\Delta(\text{araD-araB})$ 567, $\Delta(\text{lacZ4787}>::\text{rrnB-3})$, λ , $\Delta(\text{araH-araF})$ 570:: <i>FRT</i> , $\Delta\text{araEp-}$ 531:: <i>kan</i> , $\phi P_{cp8}\text{araE535}$, <i>rph-1</i> , $\Delta(\text{rhaD-}$ <i>rhaB</i>)568, <i>hsdR514</i>	2
BW27749 $\Delta ybdZ$	BW27749 in-frame deletion of <i>ybdZ</i>	this study
<i>Bacillus subtilis</i> strain 168	DNA source for BSU31959	3; J.D. Wang
<i>Pseudomonas aeruginosa</i> PAO1	DNA source for PA2412	4
<i>Agrobacterium fabrum</i> str. C58	DNA source for <i>atu3678</i>	5
<i>Myxococcus xanthus</i> DK1622	DNA source for MXAN_3118	6; L.J. Shimkets

Table S2. Sequences of primers used in this study

Primer name:	Sequence:
pBAD33_XbaI_RBS_5'PIPE	5'-ATGTATATCTCCTTCTTAAAG
pBAD33_SalI_3'PIPE	5'- GTCGACAAGCTTGCGGCCGCAC
mbtH_pBAD33_XbaI_RBS	5'- TAAGAAGGAGATATACATGTGAGCACCAATCCTTTTCGATG
mbtH_pBAD33_SalI	5'- GGCCGCAAGCTTGTCGACTCAGTCCTCGACCATGGCGTC
pa2412_pBAD33_XbaI_RBS	5'- TAAGAAGGAGATATACATATGACTTCAGTGTTTCGACCGTG
pa2412_pBAD33_SalI	5'- GGCCGCAAGCTTGTCGACTCAGCCGGCCGCCTTGTCCATG
3'_MXAN3118_pBAD	5'- AAAGGTAAATGCTGGCTCATTTCGCGAGGGGCGTGCGCACG
5'_MXAN3118_pBAD+RBS	5'- CTTTAAGAAGGAGATATACATATGACGGATGAGCGAGAGG
Atu3678_pBAD33_F	5'- TAAGAAGGAGATATACATATGAGTTCCCAGACACCGGCTGAAG
Atu3678_pBAD33_R	5'- CTGAAAATCTTCTCTCGAGCTATTCTTGCGCGCTGGTGATGTTTG
BSU31959_pBAD33_F	5'- TAAGAAGGAGATATACATATGGCAAATCCTTTTGAAAATGCGGA
BSU31959_pBAD33_R	5'- CTGAAAATCTTCTCTCGAGTTACACATTTTCAACAGTCTTTAG
cmnN_pBAD33_XbaI_RBS	5'- TAAGAAGGAGATATACATATGGACACGTACCTGGTG
cmnN_pBAD33_SalI	5'- GGCCGCAAGCTTGTCGACTCACACCGCCTCCGCGCGGCG
VioN_pBAD_R	5'- GGCCGCAAGCTTGTCGACTCATGCGCGGGCGCTGAGCGG

VioN_pBAD_F	5'- TAAGAAGGAGATATACATGAACGACACCCCTGCGGAC
PIPE_pTEV_EntF_F	5'- GAAAACCTGTATTTTCAGGGCATGAGCCAGCATTACCT
PIPE_pTEV_EntF_R	5'- GCTCGAGAATTCCATGGCATTTTCTGTAATTATGGGT
entB PIPE pTEV Forward	5'- AACCTGTATTTTCAGGGCGCTATTCCAAAATTACAGGCTTAC
entB PIPE pTEV Reverse	5'- GCTCGAGAATTCCATGGCTTATTTACCTCGCGGGAGAG
PIPE ybdZ into pTEV5 Forward	5'- AACCTGTATTTTCAGGGCGCATTCAGTAATCCCTTCGATG
PIPE ybdZ into pTEV5 Reverse	5'- GCTCGAGAATTCCATGGCTCATTGTGCCTCCTGCAACTGGG
pTEV_PIPE_PA2412__5'_	5'- GCTCGAGAATTCCATGGCTCAGCCGGCCGCCTTGTCATG
pTEV_PIPE_PA2412__3'_	5'- GAAAACCTGTATTTTCAGGGCATGACTTCAGTGTTTCGACC
pTEV_PIPE_3118_2__5'_	5'- GCTCGAGAATTCCATGGCGTCCTCGGCCATGGCGTCAC
pTEV_PIPE_3118_1_3'_	5'- GAAAACCTGTATTTTCAGGGCACGGATGAGCGAGAGGA
pTEV PIPE ATU3678	5'- AAAACCTGTATTTTCAGGGCATGAGTTCCCAGACACCGG
pTEV PIPE ATU3678-2	5'- GCTCGAGAATTCCATGGCCTATTCTTGCGCGCTGGT
pTEV PIPE BSU31959	5'- AAAACCTGTATTTTCAGGGCATGGCAAATCCTTTTGAAA
pTEV PIPE BSU31959-2	5'- GCTCGAGAATTCCATGGCTTACACATTTTCAACAGT
pTEV_PIPE_CmnN_F	5'- GAAAACCTGTATTTTCAGGGCATGGACACGTACCTGGTGG
pTEV_PIPE_CmnN_R	5'- GCTCGAGAATTCCATGGCTCACACCGCCTCCGCGCG
pTEV_VioN5'	5'- AAAACCTGTATTTTCAGGGCATGAACGACACCCCTGCGGA
pTEV_VioN3'	5'- GCTCGAGAATTCCATGGCTGAAAATCTTCTCTCATCCG

pTEV PIPE 1	5'- GCCTGAAAATACAGGTTTT
pTEV PIPE2	5'- GCCATGGAATTCTCGAGC
pTEV_AScan_Seq	5'- CGACTCACTATAGGGGAATT
pACYCDuet_1_NcoI_5	5'- CATGGTATATCTCCTTATTAAAGT
pACYCDuet_1_HindIII_3	5'- AAGCTTGCGGCCGCATAATGCTTA
PA2412-NdeI	5'- ATA AGG AGA TAT ACC ATG ACT TCA GTG TTC GAC CGT GAC
PA2412-HindIII	5'- TTA TGC GGC CGC AAG CTT TCA GAG CAT TTC CAG CTT CGA
MXAN_3118_pACYC_R	5'- TTATGCGGCCGCAAGCTTCTACGACTTCAGCTCTTCCA
MXAN_3118_pACYC_F	5'- ATAAGGAGATATACCATGACGGATGAGCGAGAGGA
3678 Duet F	5'- ATAAGGAGATATACCATGAGTTCCCAGACACCGGCTGAAG
3678 Duet R	5'- TTATGCGGCCGCAAGCTTCTATTCTTGCGCGCTGGTGATG
BSU31959_pDUET_F	5'- ATAAGGAGATATACCATGGCATGGCAAATCCTTTTGAAAATGCC GA
BSU31959_pDUET_R	5'- TTATGCGGCCGCAAGCTTTACACATTTTCAACAGTCTTTAG

Table S3. Plasmids used in this study.

Plasmids:	Purpose:	Source:
pBAD33	arabinose-inducible expression vector	7
pBAD33- <i>ybdZ</i>	expression of <i>ybdZ</i>	1
pBAD33- <i>mbtH</i>	expression of <i>mbtH</i>	this study
pBAD33-PA2412	expression of PA2412	this study
pBAD33-MXAN_3118	expression of MXAN_3118	this study
pBAD33-Atu3678	expression of Atu3678	this study
pBAD33-BSU31959	expression of BSU31959	this study
pBAD33- <i>cmnN</i>	expression of <i>cmnN</i>	this study
pBAD33- <i>vioN</i>	expression of <i>vioN</i>	this study
pACYC duet-1	T7 coexpression vector	1
pACYC duet- <i>ybdZ</i>	coexpression of <i>ybdZ</i>	1
pACYC duet- <i>mbtH</i>	coexpression of <i>mbtH</i>	8
pACYC duet-PA2412	coexpression of PA2412	this study
pACYC duet-MXAN_3118	coexpression of MXAN_3118	this study
pACYC duet-Atu3678	coexpression of Atu3678	this study
pACYC duet-BSU31959	coexpression of BSU31959	this study
pACYC duet- <i>cmnN</i>	coexpression of <i>cmnN</i>	1

pACYC duet- <i>vioN</i>	coexpression of <i>vioN</i>	1
pTEV5	T7 overexpression vector, TEV protease cleave site	9
pTEV5- <i>entF</i>	overexpression of <i>entF</i>	this study
pTEV5- <i>ybdZ</i>	overexpression of <i>ybdZ</i>	this study
pTEV5- <i>mbtH</i>	overexpression of <i>mbtH</i>	8
pTEV5-PA2412	overexpression of PA2412	this study
pTEV5-MXAN_3118	overexpression of MXAN_3118	this study
pTEV5-Atu3678	overexpression of Atu3678	this study
pTEV5-BSU31959	overexpression of BSU31959	this study
pTEV5- <i>cmnN</i>	overexpression of <i>cmnN</i>	this study
pTEV5- <i>vioN</i>	overexpression of <i>vioN</i>	this study
pTEV5- <i>entB</i>	overexpression of <i>entB</i>	this study
pSU20- <i>sfp</i>	T7 coexpression vector, expressing <i>sfp</i>	10
pMAK705- <i>ybdZ</i>	Temperature sensitive plasmid containing <i>ybdZ</i>	1

Table S4. Gene insert sequences for construction of MLP-T7 constructs used to investigate *in vivo* levels of MLPs under induction.

<p>ybdZ-T7 (XbaI/HindIII)</p>	<p>ATCTAGAAGGAGATATACATATGGCATTTCAGTAA TCCCTTCGATGATCCGCAGGGAGCGTTTTACATA TTGCGCAATGCGCAGGGGCAATTCAGTCTGTGG CCGCAACAATGCGTCTTACCGGCAGGCTGGGAC ATTGTGTGTCAGCCGCAGTCACAGGCGTCCTGCC AGCAGTGGCTGGAAGCCCACTGGCGTACTCTGA CACCGACGAATTTTACCCAGTTGCAGGAGGCAC AAATGGCTAGCATGACTGGTGGACAGCAAATGG GTTGAAGCTTAACTCGAGAGAAGATTTTCAGCCT GATAC</p>
<p>mbtH-T7 (XbaI/HindIII)</p>	<p>AATCTAGAAGGAGATATACATATGAGCACCAAT CCTTTCGATGACGACAACGGCGCATTCTTCGTGC TGGTCAACGACGAAGACCAGCACAGCCTGTGGC CGGTGTTCCCGATATCCCGGCCGGCTGGCGCG TGGTGCACGGCGAAGCCAGCCGTGCCGCCTGCC TGGACTACGTGGAAAAGAACTGGACCGATCTGC GGCCGAAGAGCCTGCGTGACGCCATGGCCGAG GACATGGCTAGCATGACTGGTGGACAGCAAATG GGATGAAGCTTAACTCGAGAGAAGATTTTCAGC CTGATAC</p>

<p>3118-T7 (XbaI/HindIII)</p>	<p>AATCTAGAAGGAGATATACATATGACGGATGAG CGAGAGGACACGACCGTCTACAAGGTCGTGGTG AACCACGAGGAGCAGTACTCCATCTGGCCGGCC GACCGCGAGAACGCGCTCGGCTGGAAGGATGC AGGCAAGCAGGGCCTCAAGGCCGAGTGCCTGG AGTACATCAAGGAGGTCTGGACGGACATGCGTC CGCTGAGCCTCCGCAAGAAGATGGAAGAGCTGA AGTCGATGGCTAGCATGACTGGTGGACAGCAAA TGGGATGAAGCTTAACTCGAGAGAAGATTTTCA GCCTGATAC</p>
<p>PA2412 gblock</p>	<p>ATCTCTAGAAAATAATTTTGTTTAACTTTTAAGAA GGAGATATACCATATGACTTCAGTGTTTCGACCGT GACGACATCCAGTTCAGGTAGTGGTCAACCAT GAGGAGCAGTATTCCATCTGGCCGGAATACAAG GAGATTCCCCAGGGCTGGCGGGCGGCCGGCAA GAGCGGCCTGAAGAAGGACTGCCTGGCCTACAT CGAGGAAGTCTGGACCGACATGCGCCCGCTGAG CCTGCGCCAGCACATGGACAAGGCCGGCCGGCAT GGCTAGCATGACTGGTGGACAGCAAATGGGTTG ATCGAGCTGCAGGCATGCAAGCTTGGCTGTTTTG GCGGATGAG</p>

<p>BSU31959 gblock</p>	<p>ATCTCTAGAAAATAATTTTGTTTAACTTTTAAGAA GGAGATATACCATATGGCAAATCCTTTTGAAAAT GCGGATGGCACATATTTGGTGCTGGTCAATGAA GAAGGCCAATATCCCTATGGCCGGGTTTTATAG ATGTGCCGAGCGGCTGGACAGTCGTTTCATGAGC AAAAAGGGCGTGAAGCTTGTGGACTATATCC AATCGCATTGGAGCGATATGAGGCCAAACAGCC TAAAGACTGTTGAAAATGTGATGGCTAGCATGA CTGGTGGACAGCAAATGGGTTGATCGAGCTGCA GGCATGCAAGCTTGGCTGTTTTGGCGGATGAG</p>
<p>Atu3678 gblock</p>	<p>ATCTCTAGAAAATAATTTTGTTTAACTTTTAAGAA GGAGATATACCATATGAGTTCCCAGACACCGGCT GAAGACCTGCACTACAACGTCGTGATCAGCGAC GAAGAGCGATACTCGATATGGCCGGTCTACAAG GCCGTCCCGGCGGGGTGGCGATTGAGCGGCTTT TCCGGATCGAAGCAGGCGTGCCTCGATCATATC GAGGTGGAGTGGACCGACATGCGCCCCTTGAGC TTGCGGCGGTTGATGGACGGCGAAGCAGCAAAC ATCACCAGCGCGCAAGAAATGGCTAGCATGACT GGTGGACAGCAAATGGGTTGATCGAGCTGCAG GCATGCAAGCTTGGCTGTTTTGGCGGATGAG</p>

<p align="center">CmnN gblock</p>	<p>ATCTCTAGAAAATAATTTTGTTTAACTTTTAAGAA GGAGATATAACCATATGGACACGTACCTGGTGGT CGTCAACCACGAGGAGCAGTACTCGGTGTGGCC GGCCGACCGCCGCTGCCCGCCGGTGGCGTGC CGAGGGCACGTCCGGCGACAAGGAGCAGTGCCT CGCGCACATCGAGACCGTGTGGACCGACATGCG CCCGCTCAGCGTGCGCCGCCGCGCGGAGGCGGT GATGGCTAGCATGACTGGTGGACAGCAAATGGG TTGATCGAGCTGCAGGCATGCAAGCTTGGCTGTT TTGGCGGATGAG</p>
<p align="center">VioN gblock</p>	<p>ATCTCTAGAAAATAATTTTGTTTAACTTTTAAGAA GGAGATATAACCATATGAACGACACCCCTGCGGA CACCGCGTACCAGGTCGTCCTGAACGACGAGGA GCAGTACTCCGTGTGGCCGGTGGGCCGCGCT CCCGGCCGCTGGCGGGCCGAGGGCACTGTCTG GTGGCCGCCAGGCGTGCCTGGACCACATCGAGA CGGTCTGGACCGACCTGCGTCCGCTCAGCGCCC GCGCAATGGCTAGCATGACTGGTGGACAGCAA TGGGTTGATCGAGCTGCAGGCATGCAAGCTTGG</p>

Table S5. Elemental formulae, calculated and measured m/z of ENT and biosynthetic intermediates.

	Elemental formula	Measured m/z	Calculated m/z
ENT	C ₃₀ H ₂₇ N ₃ O ₁₅	668.1356	668.1442
(DHB-L-Ser) ³	C ₃₀ H ₂₉ N ₃ O ₁₆	686.1462	686.1548
(DHB-L-Ser) ²	C ₂₀ H ₂₀ N ₂ O ₁₁	463.0985	463.1067
DHB-L-Ser	C ₁₀ H ₁₁ NO ₆	240.0509	240.0586

References:

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